HHS Public Access

Author manuscript

Indiana Univ J Undergrad Res. Author manuscript; available in PMC 2019 March 20.

Published in final edited form as:

Indiana Univ J Undergrad Res. 2018 ; 4(1): 17–26. doi:10.14434/iujur.v4i1.24499.

Systematic Survey of the Role of IGF in the Link Between Diabetes and Cancer

Nirupama Devanathan and **Dr. Ann C. Kimble-Hill [Faculty Mentor]**

Indiana University School of Medicine

Abstract

Epidemiological studies have proposed a link between type II diabetes and cancer via the IGF/insulin signaling pathway, which includes insulin-like peptides (IGF1, IGF2, and insulin), insulin receptors (IR-A, IR-B, IGF1R, and hybrids), and insulin substrate proteins (IRS1–6). In this study, up- and down-regulation of various components in the IGF/insulin signaling pathway are compared to clinical outcomes for cancer patients; the components include diagnosis age, overall survival, tumor invasion and vascularization, and body mass index. It was found that the up-regulation of insulin growth Factor (IGF)/insulin components was associated with overall survival and tumor invasion and vascularization, while the down-regulation of equivalent components was not associated with clinical outcomes assessed in this study. Particularly, the up-regulation of DOK5, IGF2, and IRS2 in colorectal cancer and IGF1R in liver cancer is associated with significantly decreased overall survival. Functional aberrations in either of the two proteins in co-expression pairs were identified for each cancer and correlated with overall survival and diagnosis age. Specific biomarkers proposed in this study will be further analyzed to fine-tune consistent associations that can be translated to reliable prognostic standards for the roles of IGF/insulin signaling pathway modulations that promote cancer.

Keywords

Type II diabetes (T2DM); colorectal cancer; liver cancer; pancreatic cancer; uterine cancer; insulin growth factor (IGF); insulin receptor (IR)

INTRODUCTION

Type II diabetes mellitus (T2DM) and cancer of the liver, pancreas, endometrium, colorectal, breast, and bladder share a number of epidemiological trends (Arcidiacono et al., 2012a; Giovannucci et al., 2010; Vigneri, Frasca, Sciacca, Pandini, & Vigneri, 2009). Traditionally associated with industrialized nations, cancer and T2DM are growing epidemics in lower- and middle-income nations. By 2025, 20 million new cancer cases are predicted, with the greatest incidence in the developing world (Ferlay et al., 2015). Of even greater concern, the global population of people living with T2DM continues to soar. By 2030, 440 million people are expected to develop T2DM, with the greatest prevalence occurring in the densely populated nations of India, China, and Bangladesh (Chen, Magliano, & Zimmet, 2011).

All correspondence should be sent to the senior author: ankimble@iu.edu.

Apart from documenting this demographic shift—a pronounced increase in cancer and T2DM in less developed nations— epidemiological studies have also proposed T2DM as a risk factor to cancer development, implicating shared molecular connections (Arcidiacono et al., 2012b; Vigneri et al., 2009). Hyperinsulinemia, an early onset indicator for T2DM prognosis, disrupts the insulin growth factor (IGF)/insulin signaling pathway, which, in turn, perturbs key signaling pathways that enable cancer cell viability (Arcidiacono et al., 2012a; Bowers, Rossi, O'Flanagan, deGraffenried, & Hursting, 2015; Djiogue et al., 2013). Furthermore, T2DM hyperglycemic conditions favor cancer's tendency to metabolize via the highly inefficient process of glycolysis—a phenomenon dubbed as the Warburg Effect—by providing an extreme surplus in blood glucose (Orgel & Mittelman, 2013).

Consequently, it has been proposed that the IGF/insulin signaling pathway serves as another molecular link between T2DM and cancer (Djiogue et al., 2013).

Insulin/IGF Signaling Pathway Proteins Identified and Their Roles

There are three areas of IGF/insulin signaling that may drive this relationship: receptor activators such as insulin and IGF; insulin receptor (INSR), insulin growth factor receptor (IGFR), and their heterodimers; and the ligands insulin receptor substrate (IRS), IGF binding protein (IGFBP), and docking protein (DOK). Insulin and IGF are classified as insulin-like peptides which are crucial in the regulation of energy metabolism (Bowers et al., 2015; Djiogue et al., 2013). Insulin is produced exclusively in the pancreas via beta cells in the islets in response to sensing glucose in the blood stream. Insulin plays an essential role in the progression of IGF/insulin signaling, as it activates both isoforms of INSR (IR-A and IR-B). The expression of IR-A is primarily found in metabolic tissues, including the liver (Bowers et al., 2015). In the insulin/IGF system, IR-B is insulin-specific and is exclusively involved with glucose homeostasis (Djiogue et al., 2013).

IGF is primarily produced in the liver but also generated locally by tumor cells. Growth hormone stimulates IGF production in the liver, but IGF can also be produced by cancer cells themselves, thereby generating a microenvironment suitable for maintaining cellular function in dense tumors where cells face a reduction in insulin stimulation. IGF1 has been known to stimulate INSR signaling, as IR-B has been shown to form heterodimers with IGF (Bowers et al., 2015). IGF2 is also able to activate IR-A and is found in all adult tissue types (Djiogue et al., 2013). Additionally, IGFIR heterodimers have been reported to be found in cancerous cells (Djiogue et al., 2013). These receptors typically auto-phosphorylate at the beta-subunit tyrosine kinase domains (Cohen & LeRoith, 2012); then, the receptors are able to activate INSR.

IGFBP regulates the relative bioavailability of IGF (Baxter, 2014), as hyperinsulinemia increases IGF1 bioavailability through the suppression of IGFBP synthesis, promotion of oxidative stress, increases in pro-inflammatory cytokines, and promotion of growth hormone receptor in the liver, which allows for greater IGF1 synthesis (Arcidiacono et al., 2012b; Bowers et al., 2015).

While previous studies have attempted to correlate the different serum levels associated with components of IGF/insulin signaling, factors like variable bioavailability have rendered such

measures to be less useful (Cohen & LeRoith, 2012). In this study, cancerspecific DNA, mRNA, and protein expression data from patients that have liver, pancreatic, uterine, and colorectal cancer were obtained from the cBIO Database Portal datasets. Next, genomic and proteomic expression changes to components of the IGF/insulin signaling pathway were related to risk factors and clinical outcomes for cancer patients. Importantly, this study presents associations between clinical outcomes, up- and down-regulated components of IGF/insulin signaling, and candidate pro-cancer biomarkers for future investigation.

METHODOLOGY

All data in this study were collected using the cBIO Database Portal, particularly depending on The Cancer Genome Atlas (TCGA) provisional studies, as of October 15th, 2017 (Cerami et al., 2012; Gao et al., 2013). We obtained DNA mutation, mRNA expression, and protein expression level data for the following tumor types previously reported to have epidemiologically significant association with type II diabetes: liver, uterine, pancreatic, and colorectal (Arcidiacono et al., 2012b). Table 1 lists the number of patient samples for each tumor type, along with the samples related to changes in IGF/insulin signaling in this analysis.

Data Collection and Processing

We used data available from TCGA to analyze up- and down-regulation of IGF/insulin signaling protein activity (e.g., IGF1, IGF2, INS, IRS1, IRS2, IRS4, DOK4, DOK5, IGF1R, INSR, IGFBP3), defined as $-2.5 \leq Z \leq 2.5$ for both mRNA and protein levels, in the previously listed cancers. In addition to explicit over- and under-expression data, up- and down-regulation was deduced using mutation information (amplification, function depression mutation, or deletion of protein). Function depression mutations were defined as those reported to lead to a loss of function (nonsense mutations or frameshift mutations) or missense mutations predicted as possibly or probably damaging to function (Gao et al., 2013). In turn, OncoPrint visualization was used to relate each mutation or annotation to the following clinical factors: diagnosis age, overall survival months, overall survival status, patient height, patient weight, and vascular invasion. The body mass index (BMI), a biometric to track obesity, was calculated for available data sets that contained patient height and weight using the following formula: $BMI = mass / height^2$.

Statistical Analysis

Each instance of mutation or annotation was categorized as either up- or down-regulated, based on the criteria mentioned above. A two-tailed t-test was conducted to compare over- and under-expression for each component of IGF/insulin signaling with the previously mentioned clinical factors. Of particular importance, survival rates— statistically determined to be significantly associated with the up-regulation of IGF1R, IGF2, IRS2, and DOK5— were analyzed through measures of central tendency, culminating with corresponding box-and-whisker plots. Box-and-whisker plots systematically divide the spread of data into quartiles, which are essential in developing a precise sense of survivability associated with significant changes in the expression of IGF/insulin signaling components.

Apart from the analysis of up- and down-regulation of IGF/insulin signaling components, this study also examined pairs of co-occurring mutations within the system. Internal algorithms associated with the cBio Portal were applied to discover the statistically significant co-occurrences ($p < 0.05$). The pairs consistently found across multiple cancer types were then proposed as candidate biomarkers to indicate the possible presence of cancer or the start of cancer formation in aberrant IGF/insulin conditions, such as hyperinsulinemia. A two-tailed t-test was then conducted to analyze possible relationships between the 1) simultaneous up-regulation, 2) simultaneous down-regulation, or 3) the up-regulation/down-regulation of the functional aberrancies in each co-occurrence pair and the clinical factors of overall survival and diagnosis age.

RESULTS

Associations that Drive Liver Cancer Patient Outcomes

As shown by the OncoPrint view diagram in Figure 1, less than 10% of mutations within the sample set were associated with IGF/insulin signaling, representing a small subset of the total cancer cases. Within this subset, pronounced patterns of amplification and mRNA up-regulation were observed, while deep deletion and missense mutations seemed to occur only a few times. In general, the highest number of changes was found to affect DOK5, which represented 5% of total samples. The remaining changes, in descending order of percent of total samples computed by the cBIO Portal, included: IGF1R (4%), INSR (4%), IRS1 (4%), IRS4 (4%), IGF2 (2.4%), DOK4 (2.2%), IRS2 (2.2%), INS (2%), IGF1 (1.5%), and IGFBP3 (1.3%) (Figure 1). IGF1R and IGFBP3 were found to experience simultaneous mutation or change in expression ($p = 0.045$).

After determining the changes in expression or mutations known within the insulin/IGF pathway for these tissues, the effect of these changes on clinical outcomes was determined (Table 6). Vascular invasion was extremely minimal, with either an absence of invasion or micro-invasion reported in nearly all of the samples. Out of the 123 samples in which information for vascular invasion was given, macrovascular invasion was reported in 30% of samples (Figure 1). The up-regulation of IRS2 and DOK5, both insulin receptor substrates, was correlated with absent or reduced vascular invasion. The up-regulation of IGF1R was correlated with lowered overall survival and increased BMI but with absent or reduced vascular correlation as well. For IGF1R, only 9% of the cases experienced vascularization, IRS2 17% of the cases, and DOK5 42% of the cases (Figure 1). While the up-regulation of IGF1R does little to induce vascularization and/or tumor invasion, it is possible that the perturbation of the substrate proteins negatively impacts the success of tumor metastasis.

Associations that Drive Pancreatic Cancer Patient Outcomes

Pancreatic ductal adenocarcinoma (PDAC) bears little resemblance to the other three cancers with regards to co-occurrence patterns. Within the PDAC subset, pronounced patterns of amplification and mRNA up-regulation were observed, while deep deletion and missense mutations seemed to occur only a few times in this dataset. The OncoPrint view diagram (Figure 2) illustrates that less than 10% of mutations within the sample set were associated with IGF/insulin signaling, representing a small subset of the total cancer cases.

Qualitatively, there was a wide variety of mutation and annotation types represented in this subset—including missense mutation, deep deletion, amplification, and mRNA up-regulation—with no one particular type of perturbation predominating. A similarly wide variety of tumor invasion was also observed in the OncoPrint. In general, the highest number of changes in pancreatic cancer was found to affect IRS1, which represented 8% of total samples. The remaining changes, in descending order of percent of total samples, included: IRS2 (6%), IRS4 (6%), IGF1R (5%), DOK4 (4%), DOK5 (4%), IGFBP3 (3%), INSR (3%), INS (1.6%), IGF1 (1.6%), and IGF2 (0.5%) (Figure 2).

Associations that Drive Uterine Cancer Patient Outcomes

The OncoPrint view diagram (Figure 3) illustrates that less than 15% of mutations within the sample set were associated with IGF/ insulin signaling, representing a small subset of total cancer cases. In general, the highest number of changes in uterine cancer was found to affect DOK5, which represented 11% of total samples. The remaining changes, in descending order of percent of total samples, included: IGF2 (6%), INS (3%), IRS2 (3%), IRS4 (2.9%), IGF1R (2.4%), INSR (2.4%), IRS1 (2.7%), IGFBP3 (1.7%), IGF1 (1.1%), and DOK4 (0.8%) (Figure 3). Vascular invasion was extremely minimal, with either an absence of invasion or micro-invasion reported in nearly all of the samples. We found a significant co-occurrence ($p < 0.001$) between the ligands IGF2/INS; receptors/substrates INSR/ IRS1, INSR/IRS4, and IGF1R/IRS4; and substrates IRS1/IRS4 and IGFBP3/IRS4 (Table 4).

Associations that Drive Colorectal Cancer Patient Outcomes

The colorectal cancer OncoPrint diagram illustrates that less than 15% of mutations within the sample set were also associated with IGF/insulin signaling (Figure 4). In general, the highest number of changes was found to affect DOK5, which represented 11% of total samples. The remaining changes, in descending order of percent of total samples, included: IGF2 (6%), IRS2 (3%), INS (3%), IRS4 (2.9%), IRS1 (2.7%), INSR (2.4%), IGF1R (2.4%), IGFBP3 (1.7%), IGF1 (1.1%), and DOK4 (0.8%) (Figure 4).

In colorectal cancer, the up-regulation of DOK5 and IRS2 was associated with overall survival status, while in liver cancer, up-regulation was associated with increased vascular invasion. On the other hand, the up-regulation of different IGF/insulin signaling components can lead to similar clinical outcomes; in colorectal cancer, the up-regulation of IGF1R, along with its adaptor proteins, is associated with overall survival. Additionally, the up-regulation of DOK5, IGF2, and IRS2 was associated with clinical outcomes of decreased average months of survival (Table 6). The median survival was approximately 25 months. Without the up-regulation of components in IGF/insulin signaling, the median survival was 83 months (Figure 5).

DISCUSSION

An association between the up-regulation of most components in IGF/insulin signaling and overall survival was observed from the two-tailed t-tests. Down-regulation in IGF/insulin signaling did not appear to be associated with clinical outcomes. Associations between specific components of IGF/insulin signaling and patient outcomes were unable to be

obtained for either uterine or pancreatic cancers due to exceedingly small sample sizes ($n < 10$). Despite relatively small sample sizes, these components were vigorously analyzed both in co-occurrence pairs and individually relative to clinical outcomes. Based on putative molecular connections underlying these relationships, we were then able to propose indicative mechanisms.

Analysis of Associations that Drive Liver Cancer Patient Outcomes

The up-regulation of IGF1R was correlated to decreased survival. In liver cancer, the up-regulation of IGF1R was associated with a median overall survival of just over 15 months, compared to 49 months for patients with normal IGF/insulin signaling (Figure 6).

We saw expected correlations between known receptor/binding protein pairs IGF1R/IGFBP3 (Table 2). A two-tailed t-test between patients that had aberrations in either IGF1R or IGFBP3 activity demonstrated that there is no statistical difference in the overall diagnosis age ($p = 0.627$), suggesting that up-regulation at either the receptor or binding protein levels demonstrates a statistically equivalent impact on the overall diagnosis age. In contrast, a statistically significant difference between the up-regulation of these two components in terms of overall survival time ($p = 0.0255$) was observed. When alterations in IGF1R and IGFBP3 co-occur, the relative activity within the pair in relation to overall cancer survival is shown to be different. The up-regulation of IGF1R has been associated with increased proliferation and overall cell survival, and correspondingly, decreased overall survival by promoting disease progression. On the other hand, while literature has related the down-regulation of IGFBP3 to tumor suppressing functionality, the impact of IGFBP3 up-regulation requires further investigation (Rebhan, Chalifa-Caspi, Prilusky, & Lancet, 1998). Aberrancies in binding proteins like IGFBP3, which control the relative bioavailability of insulin-like peptide, affect the receptor and receptor substrate proteins, which, in turn, participate in crucial segments of cancer-inducing cell signaling.

Analysis of Associations that Drive Pancreatic Cancer Patient Outcomes

Due to the small sample size of patients with changes in expression, we could not determine the effects of genomic and/or proteomic changes in IGF/insulin signaling on clinical outcomes with confidence. As illustrated in Table 3, DOK4 and INSR was the only IGF/insulin signaling pair that showed significant co-occurrence in pancreatic cancer. This correlation suggests that DOK4 is the most likely candidate to propagate the INSR signal in pancreatic cancer; however, it may have no clinical relevance for patient outcomes. These results also suggest that pancreatic tumor metabolism does not mirror that of liver tumor metabolism.

Analysis of Associations that Drive Uterine Cancer Patient Outcomes

We then assessed whether functional connections remained the same between the co-occurrence pairs identified in uterine cancer. We performed two-tailed t-tests on patient diagnosis age and survival to determine the relationship between having functional aberrations in either of the two proteins in the pairs identified.

Particularly in uterine cancer, the up-regulation of one gene and the down-regulation of the other within the co-occurrence pair proved to be significant in several cases. For instance, a statistically significant difference was found between the down-regulation of IRS4 and the up-regulation of IGF1R in terms of overall survival ($p = 0.08$) and overall diagnosis age ($p = 0.001$), respectively. We found a statistically significant difference in the simultaneous up-regulation or simultaneous down-regulation of both IRS1 and IGF1R in relation to overall survival months ($p=0.001$). We also found a statistically significant difference in relation to overall survival in months for the following pairs: 1) up-regulation of DOK4 and DOK5 ($p = 0.05$); 2) down-regulation of DOK4 and DOK5 ($p = 0.01$); 3) up-regulation of DOK4 and down-regulation of DOK5 ($p = 0.09$); and 4) down-regulation of DOK4 and up-regulation of DOK4 ($p = 0.02$).

On the other hand, no statistically significant difference was noted between the down-regulation of IRS1 and the up-regulation of IRS4 ($p = 0.08$) relative to overall patient survival. We found no statistical difference in the up-regulation of IGF1 and IRS2 ($p = 0.9$) or when IGF1 is up-regulated and IRS2 is down-regulated ($p = 0.1$), relative to diagnosis age. Similarly, we found no statistically significant difference between IGF1 and IGF1R in relation to diagnosis age, either when IGF1 is up-regulated and IGF1R is down-regulated ($p = 0.8$) or when both are simultaneously up-regulated ($p = 0.5$); in addition, we did not find a statistically significant difference between the down-regulation of IRS1 and the up-regulation of IGF1R, relative to diagnosis age ($p = 0.1$). We found no statistically significant difference between IGF1 and IRS4 in relation to diagnosis age, either when IGF1 is up-regulated and the IRS4 is down-regulated ($p = 0.8$) or when both are simultaneously up-regulated ($p = 0.9$)

As a result of determining these correlations, we began to hypothesize the role of each protein pair in uterine cancer. For instance, IRS1 has been shown to activate phosphatidylinositol 3-kinase, while IRS4 is implicated in suppressing IRS2 (Rebhan et al., 1998). Both substrate proteins, IRS1 and IRS4, act in opposition to one another, possibly accounting for the significance shown between the up- and down-regulation within the co-occurrence pair respectively (Rebhan et al., 1998). Again, as noted earlier, IRS4 is involved in the suppression of IRS1 and IRS2. The down-regulation of IRS4—which has a repressor function—along with the up-regulation of IGF1R—which will continue the signaling cascade downstream to growth pathways like the mitogen-activating protein kinase (MAPK) signaling pathway—act together to effectively promote disease progression while contributing to lower diagnosis age and overall survival respectively (Rebhan et al., 1998). As a direct adaptor protein of IGF1R with downstream activating functionality, the finding of statistical significance between simultaneous up- or down-regulation of IRS1 was surprising and requires future investigation to deduce the exact nature of this co-occurrence. As noted earlier, a statistically significant difference was found between DOK4 and DOK5 adaptor proteins that experienced either 1) simultaneous up-regulation; 2) simultaneous down-regulation; or 3) up-regulation of one member of the co-occurrence pair and down-regulation of the other. These two adaptor proteins both play a role in activating MAPK signaling, and as such, could represent a functional redundancy (Rebhan et al., 1998). The up-regulation of IGF1, as the ligand protein, remains consistent between the two co-occurrence pairs, suggesting its impact on encouraging tumor growth, regardless of the up-

or down-regulation of IGF1R. The relationship between IGF1 and IRS4 also fits this pattern in which there is a statistically significant difference between the up-regulation of IGF1 and both the up- and down-regulation of IRS4. Again, this observation underscores the impact of up-regulation at the ligand level, prompted by diabetic conditions, in promoting malignancy.

We found no difference in the diagnosis age or time of survival when either IGF1R or IRS4 is down-regulated. However, there is a slight difference between the diagnosis age when IGF1R is up-regulated compared to when IRS4 is down-regulated (~71 vs ~62 years old respectively, $p = 0.08$). For the substrates IRS1 and IRS4, there was no significant difference in diagnosis age when either was up-regulated or down-regulated, although there was a significant difference in survival time when the activity of the pairs was reciprocal to one another. Down-regulating IRS4 appears to lengthen survival time by ~25 months ($p = 0.08$). The opposing effect was seen in the other direction; however, the small sample size decreased our ability to confidently quantify this effect. We were unable to obtain large enough patient sample sizes to determine the correlations of the other co-occurrence partners. Based on the information gathered, we hypothesize that 1) IRS1 and IRS4 work in tandem, leading to the same clinical outcome of having a disease onset at an earlier age, but decreasing either lengthens the survival rate; and 2) increased IGF1R activity may reduce the effects of losing IRS4 activity, leading to onset of the disease at a later age.

Analysis of Associations that Drive Colorectal Cancer Patient Outcomes

We then looked at the co-occurrence of protein function (Table 5). We again saw connections between activators IGF/INS and substrates IRS/DOK. The dataset also demonstrated co-occurrences between the activator/receptor pair IGF/INSR and receptor/substrate pair INSR/DOK. Specifically, the statistically significant relationships included: ligand relationships between IGF2/INS ($p < 0.001$); receptor relationships between IGF1R/INSR ($p=0.05$); receptor/substrate relationships between INSR/DOK4 ($p = 0.005$) and INSR/IGFBP3 ($p = 0.03$); ligand/substrate relationships between IGF2/IRS4 ($p = 0.02$); and substrate relationships between DOK5/IRS2 ($p = 0.02$) and IGFBP3/IRS4 ($p = 0.04$). Based on these relationships, we looked at the data set more closely to determine the functional connections between these pairs.

We then performed two-tailed t-tests on patient diagnosis age and survival to determine the relationship between having functional aberrations in either of the two proteins in the pairs identified. We found no statistically significant difference between the up-regulation of IGF2 or INS in the overall diagnosis age ($p = 0.6$) or overall survival ($p = 0.7$). Similarly, the up-regulation of IGF2 and the down-regulation of INS are statistically equivalent in both overall diagnosis age ($p = 1$) but not in overall survival ($p = 0.07$). We also saw no statistically significant difference between the up-regulation of IGF2 and down-regulation of DOK5 with regards to diagnosis age ($p = 1$). We did, however, find a statistically significant difference between the up-regulation of IRS2 and DOK5 with regards to diagnosis age and overall survival ($p < 0.001$). Based on the sample size, we were unable to determine correlations between INSR/DOK4, INSR/ IGFBP3, IGFBP3/IRS4, and INSR/IGF1R.

As a result of determining these correlations, we began to hypothesize the role of each protein pair's activity in colorectal cancer. As ligands of IGF/insulin signaling, IGF2 and

INS share several receptor sites, achieved through hybridization and the versatility of the individual receptors. The statistical equivalence, therefore, is not entirely surprising (Baxter, 2014) and suggests an increased ability of these cells to respond to hyperglycemic conditions. The correlations then point to a role for downstream effects of increased receptor signaling in these conditions. DOK5, as an adaptor protein, is critical to signal transduction, especially in its interaction with the tyrosine kinase insulin receptors. The down-regulation of DOK5 would preclude its ability to regulate the downstream MAPK signaling pathway, while the up-regulation of IGF2 would also initiate downstream cell signaling without regulation. The relative equivalence of DOK5 as an adaptor and IGF2 as a ligand underscores the importance of the shared insulin receptor at the center of the pathway (Dunant, Wisniewski, Strife, Clarkson, & Resh, 2000; Rebhan et al., 1998). Both IRS2 and DOK5 are adaptor proteins and, as previously mentioned, are critical to successful signal transduction. Despite the overall similarity in function, the two proteins diverge in specific interactions. For instance, IRS2 interacts with the RET proto-oncogene, which is associated with several endocrine tumors, but DOK5 does not interact with p21, an important player in cell growth and mitogenic functionality, requiring further studies to better elucidate its role in promoting cancer progression (Dunant et al., 2000; Rebhan et al., 1998).

CONCLUSIONS

We conducted this study of using clinical observations and patient statistics to further our understanding of the correlation between T2DM and cancer of four specific tissues (liver, pancreas, uterine, and colon). Based on our statistical analyses, we found specific correlations between functional activity of proteins that initiate glucose metabolism and clinical outcomes specific to cancer type. This study suggests that the up-regulation of components in IGF/insulin signaling is associated with significantly decreased survivability, while, in contrast, down-regulation does not appear to be associated with cancer-related clinical outcomes. While different tissues shared co-occurrence pairs, evidence from this study proposes distinct patterns of functional aberrations as well as co-occurring protein pairs that could serve as biomarkers signaling potential hyperinsulinemia-induced tumorigenesis and clinical outcomes.

As such, we propose that INSR/DOK4(5), shared by uterine, pancreatic, and colorectal cancers, and IGF1R/IGFBP3, shared by uterine and liver cancers, are general biomarkers of diabetes-based cancer. Evidence demonstrates that uterine cancer can be uniquely characterized by the co-occurrences of INS/IGF2, IRS1/IRS4, INSR/ IRS1, IGFBP3/IRS4, IGF1R/IRS4, IGFBP3/IRS1, IGF1R/IGFBP3, IGF1R/INSR, IGFBP3/INSR, IGF1/IRS1, IGF1/IRS1, IGF1/IGF1R, IGF1R/IRS1, IGF1R/IRS1, IGF1/INS, DOK4/DOK5, DOK5/ IGF2, INSR/DOK5, IGF1/IRS4, and IGF1/INSR. Colorectal cancer can be characterized by the co-occurrence of IGF2/INS, IRS4/IGF2, DOK5/ IRS2, IGFBP3/INSR, IGFBP3/IRS4, and IGF1R/INSR. Subsequent studies will analyze these putative genomic biomarkers to develop reliable prognostic standards for cancers promoted by T2DM.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Rafeal Bahamonde (IUPUI) for his assistance with interpreting sample sizes in this study.

APPENDIX

Table 1.

Total Number of Samples of Each Tumor Type

Cancer Type	Number of Samples	Number of Samples with Changes Related to IGF/Insulin Signaling
Pancreatic	185	64
Uterine	113	547
Liver	440	166
Colorectal	629	177

Table 2.

Liver Cancer: Significant Co-Occurring Mutation Pairs

Gene A	Gene B	No. of Patients				Log Odds Ratio	p-Value	Tendency
		Neither	A Not B	B Not A	Both			
IGF1R	IGFBP3	394	29	15	4	1.287	0.047	Co-occurrence

Table 3.

Pancreatic Ductal Adenocarcinoma: Significant Co-Occurring Mutation Pairs

Gene A	Gene B	No. of Patients				Log Odds Ratio	p-Value	Tendency
		Neither	A Not B	B Not A	Both			
INSR	DOK4	174	4	6	2	2.674	0.022	C-occurrence

Table 4.

Uterine Cancer: Significant Co-Occurring Mutation Pairs

Gene A	Gene B	No. of Patients				Log Odds Ratio	p-Value	Tendency
		Neither	A Not B	B Not A	Both			
INS	IGF2	533	2	4	9	>3	<0.001	Co-occurrence
IRS1	IRS4	513	13	13	8	>3	<0.001	Co-occurrence
INSR	IRS1	510	17	13	7	2.708	<0.001	Co-occurrence
IGFBP3	IRS4	523	3	18	4	>3	<0.001	Co-occurrence
IGF1R	IRS4	509	17	16	6	2.418	<0.001	Co-occurrence
INSR	IRS4	508	18	16	6	2.359	<0.001	Co-occurrence
IGFBP3	IRS1	523	4	18	3	>3	0.002	Co-occurrence
IGF1R	IGFBP3	521	20	4	3	2.972	0.002	Co-occurrence
IGF1R	INSR	506	18	19	5	2.001	0.002	Co-occurrence
IGFBP3	INSR	520	4	21	3	2.922	0.002	Co-occurrence
IGF1	IRS1	522	5	18	3	2.856	0.002	Co-occurrence
IGF1	IGF1R	520	5	20	3	2.747	0.003	Co-occurrence
IGF1R	IRS1	508	19	17	4	1.839	0.009	Co-occurrence

Gene A	Gene B	No. of Patients				Log Odds Ratio	p-Value	Tendency
		Neither	A Not B	B Not A	Both			
IGF1	INS	531	6	9	2	2.979	0.01	Co-occurrence
DOK4	D0K5	513	9	22	3	2.053	0.013	Co-occurrence
DOK5	IGF2	513	22	10	3	1.945	0.018	Co-occurrence
INSR	DOK5	503	20	21	4	1.567	0.019	Co-occurrence
IGF1	IRS4	520	6	20	2	2.159	0.037	Co-occurrence
IGF1	INSR	518	6	22	2	2.06	0.044	Co-occurrence

Table 5.

Colorectal Cancer: Significant Co-Occurring Mutation Pairs

Gene A	Gene B	No. of Patients				Log Odds Ratio	p-Value	Tendency
		Neither	A Not B	B Not A	Both			
IGF2	INS	588	26	6	13	>3	<0.001	Co-occurrence
INSR	DOK4	615	13	3	2	>3	0.005	Co-occurrence
IRS4	IGF2	580	13	35	4	1.555	0.02	Co-occurrence
DOK5	IRS2	550	61	16	6	1.218	0.021	Co-occurrence
IGFBP3	INSR	609	9	13	2	2.343	0.025	Co-occurrence
IGFBP3	IRS4	606	9	16	2	2.13	0.036	Co-occurrence
IGF1R	INSR	605	13	13	2	1.968	0.046	Co-occurrence

Table 6.

Significant Clinical Outcomes Associated with the Up-Regulation of IGF/Insulin with Two-Tailed T-Test Values

Cancer	IGF/insulin Signaling Component	Sample #	Clinical Effect	p- value
Liver	↑IRS2	n=21	Vascular Invasion	<0.0001
			BMI	
	↑D0K5	n=12	Vascular Invasion	<0.0001
	↑TIGF1R	n=13	Overall Survival	<0.0001
			Vascular Invasion	
			BMI	
Colorectal	↑DOK5	n =46	Overall Survival	<0.0001
	↑TIRS2	n=13	Overall Survival	<0.0001
	↑TIGF2	n=17	Overall Survival	<0.005

REFERENCES

Arcidiacono B, Iiritano S, Nocera A, Possidente K, Nevolo MT, Ventura V, … Brunetti A (2012a). Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms. Experimental Diabetes Research, 2012.

Arcidiacono B, Iiritano S, Nocera A, Possidente K, Nevolo MT, Ventura V, … Brunetti A (2012b). Insulin Resistance and Cancer Risk: An Overview of the Pathogenetic Mechanisms. Experimental Diabetes Research, 2012, 12. doi:10.1155/2012/789174

Baxter RC (2014). IGF binding proteins in cancer: mechanistic and clinical insights. Nat Rev Cancer, 14(5), 329–341. doi:10.1038/nrc3720 [PubMed: 24722429]

Bowers LW, Rossi EL, O'Flanagan CH, deGraffenried LA, & Hursting SD (2015). The Role of the in Cancer: Lessons Learned from Clinical Trials and the Energy Balance-Cancer Link. Front Endocrinol (Lausanne), 6, 77. doi:10.3389/fendo.2015.00077 [PubMed: 26029167]

Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, … Schultz N (2012). The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. Cancer Discovery, 2(5), 401–404. doi:10.1158/2159-8290.cd-12-0095 [PubMed: 22588877]

Chen L, Magliano DJ, & Zimmet PZ (2011). The worldwide epidemiology of type 2 diabetes mellitus —present and future perspectives. Nature Reviews Endocrinology, 8, 228. doi:10.1038/nrendo.2011.183

Cohen DH, & LeRoith D (2012). Obesity, type 2 diabetes, and cancer: the insulin and IGF connection. Endocrine-Related Cancer, 19(5), F27–F45. doi:10.1530/erc-11-0374 [PubMed: 22593429]

Djiogue S, Nwabo Kamdje AH, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, & Seke Etet PF (2013). Insulin resistance and cancer: the role of insulin and IGFs. Endocrine-Related Cancer, 20(1), R1–R17. doi:10.1530/erc-12-0324 [PubMed: 23207292]

Dunant NM, Wisniewski D, Strife A, Clarkson B, & Resh MD (2000). The phosphatidylinositol polyphosphate 5-phosphatase SHIP1 associates with the Dok1 phosphoprotein in Bcr-Abl transformed cells. Cellular Signalling, 12(5), 317–326. doi:10.1016/S0898-6568(00)00073-5 [PubMed: 10822173]

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, … Bray F (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 136(5), E359–386. doi:10.1002/ijc.29210 [PubMed: 25220842]

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, … Schultz N (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal, 6(269), pl1. doi:10.1126/scisignal.2004088

Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, … Yee D (2010). Diabetes and Cancer: A Consensus Report. CA: A Cancer Journal for Clinicians, 60(4), 207–221. doi:10.3322/caac.20078 [PubMed: 20554718]

Orgel E, & Mittelman SD (2013). The Links Between Insulin Resistance, Diabetes, and Cancer. Current Diabetes Reports, 13(2), 213–222. doi:10.1007/s11892-012-0356-6 [PubMed: 23271574]

Rebhan M, Chalifa-Caspi V, Prilusky J, & Lancet D (1998). GeneCards: a novel functional genomics compendium with automated data mining and query reformulation support. Bioinformatics, 14(8), 656–664. doi:10.1093/bioinformatics/14.8.656 [PubMed: 9789091]

Vigneri P, Frasca F, Sciacca L, Pandini G, & Vigneri R (2009). Diabetes and cancer. Endocrine-Related Cancer, 16(4), 1103–1123. doi:10.1677/erc-09-0087 [PubMed: 19620249]

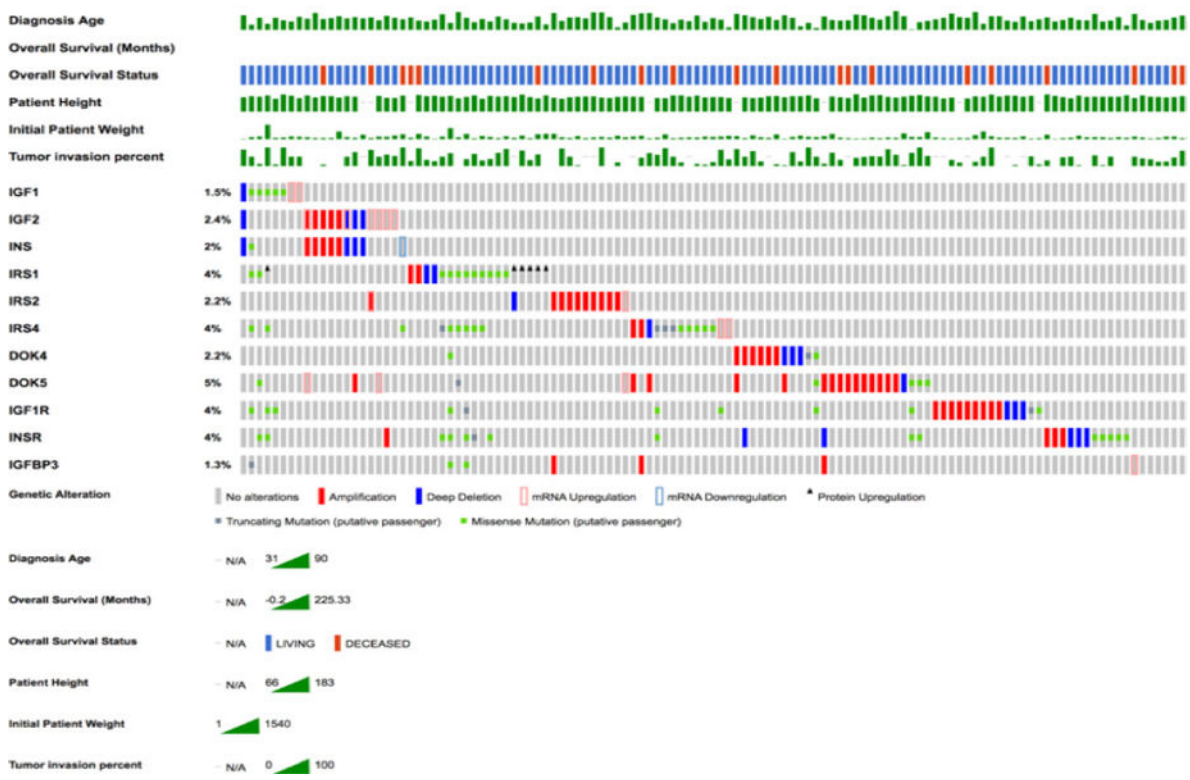

Figure 1. OncoPrint View of Liver Cancer.

The mutations and annotations for the respective samples in liver cancer are presented, along with corresponding clinical outcomes: diagnosis age, overall survival in months, overall survival status, patient height, patient weight, and vascular invasion.

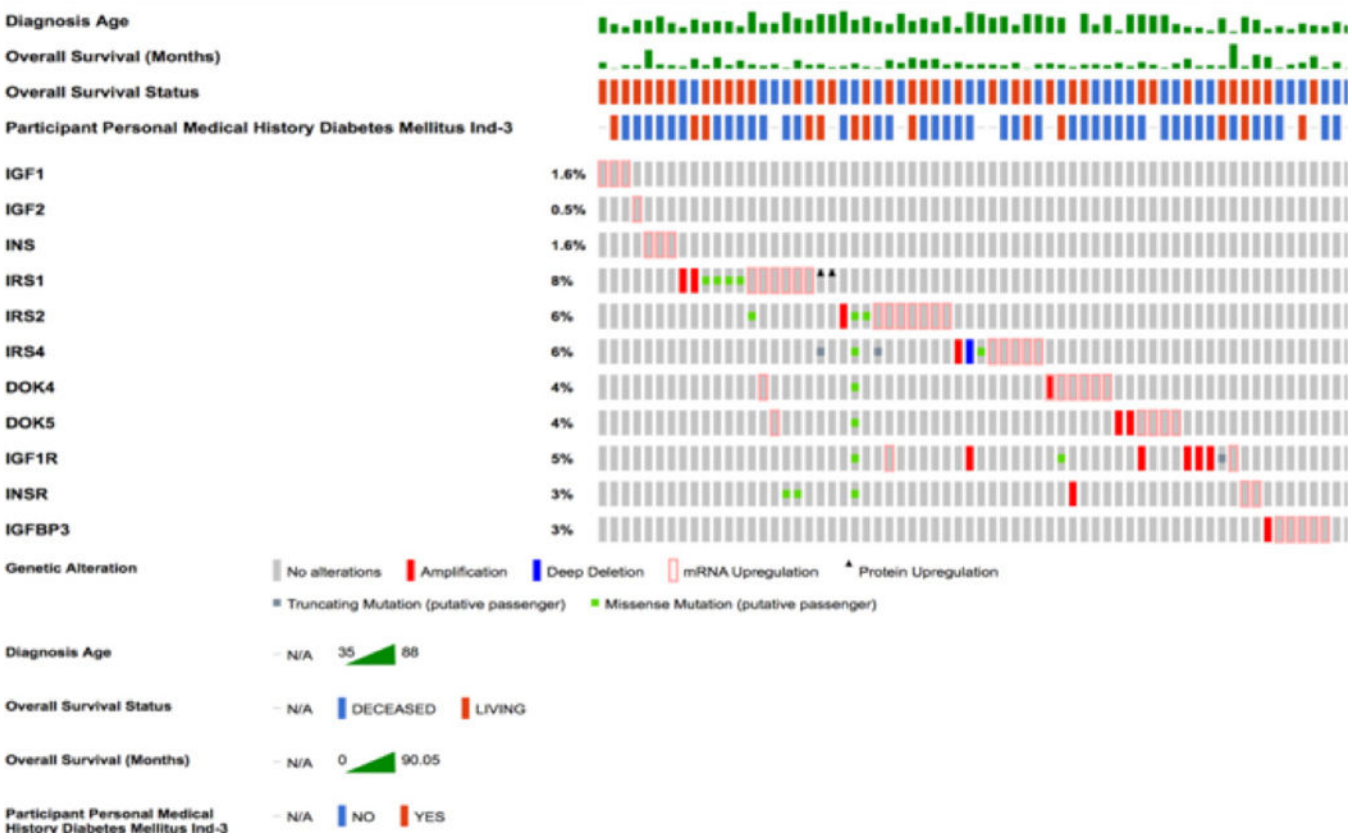

Figure 2. OncoPrint View of Pancreatic Cancer.

The mutations and annotations for the respective samples in pancreatic cancer are presented, along with corresponding clinical outcomes: diagnosis age, overall survival in months, overall survival status, patient height, patient weight, and tumor invasion percent.

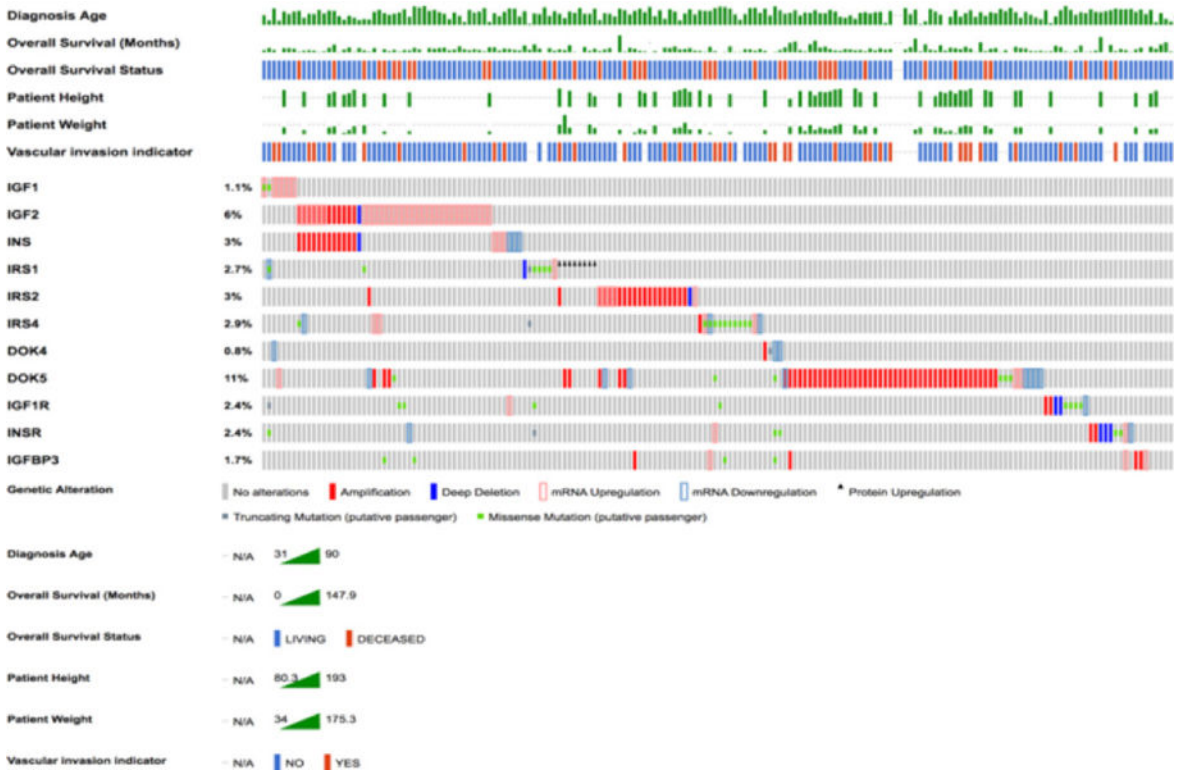

Figure 3. OncoPrint View of Uterine Cancer.

The mutations and annotations for the respective samples in uterine cancer are presented, along with corresponding clinical outcomes: diagnosis age, overall survival in months, overall survival status, and personal medical history of T2DM.

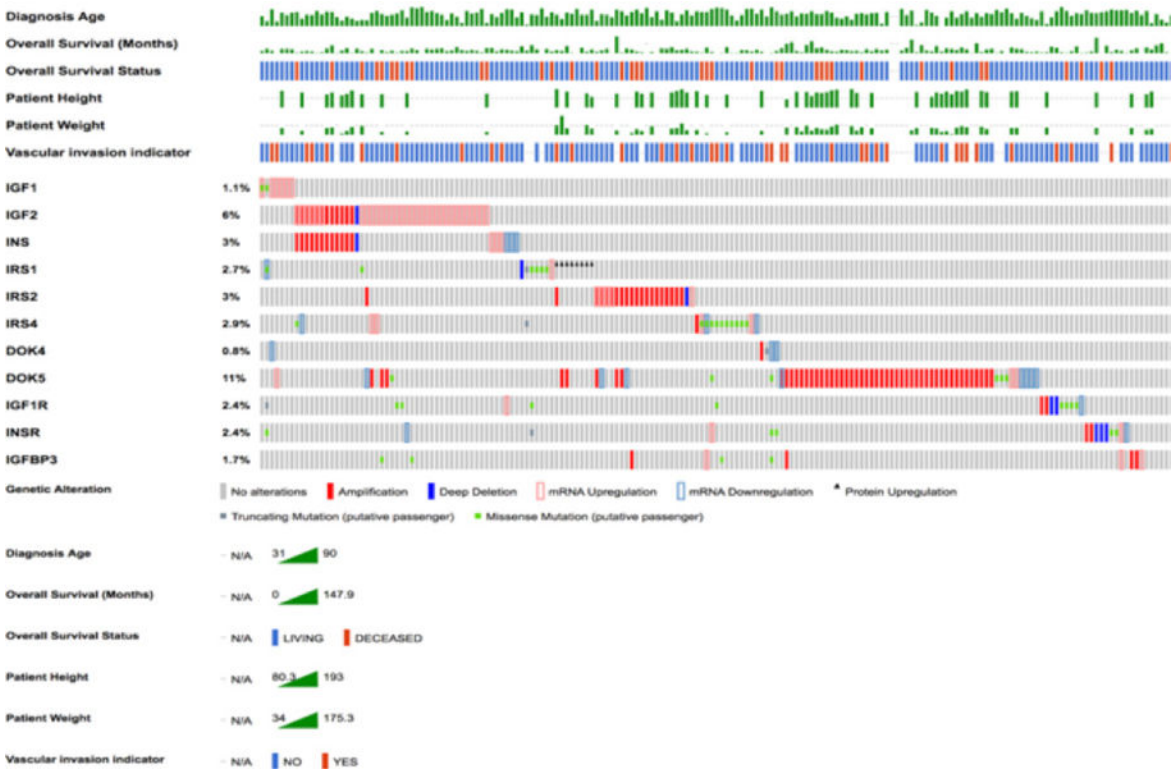

Figure 4. OncoPrint View of Colorectal Cancer.

The mutations and annotations for the respective samples in colorectal cancer are presented, along with corresponding clinical outcomes: diagnosis age, overall survival in months, overall survival status, patient height, patient weight, and vascular invasion. Note that patient height and weight data are largely unavailable in this study.

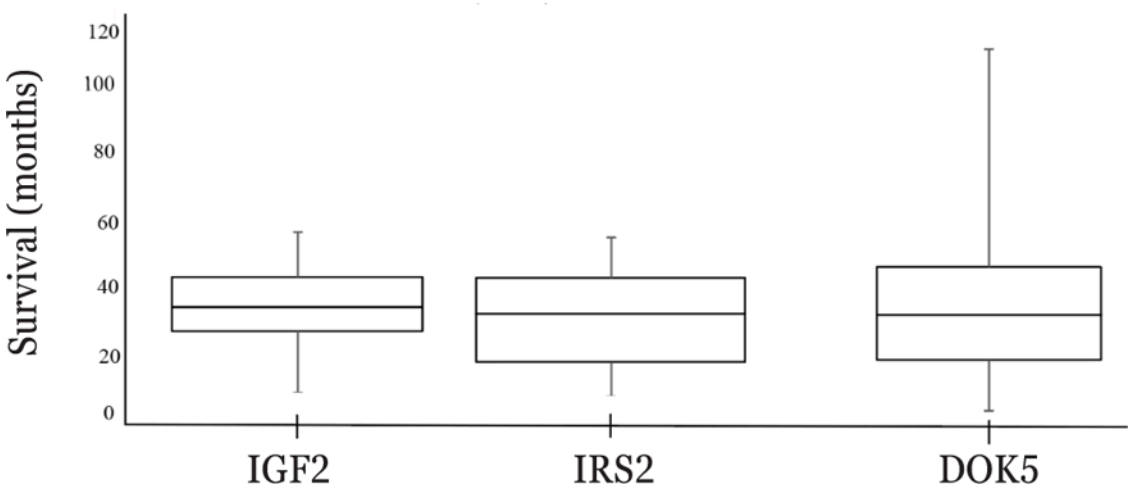

Figure 5. Survival (Months) for IGF2, IRS2, and DOK5 in Colorectal Cancer.

Three box-and-whisker plots are given to compare the spread of patient survival data in colorectal cancer and particularly emphasize the low median survival for each observed component. Samples with the up-regulation of DOK5 exhibit the greatest range of survival outcomes.

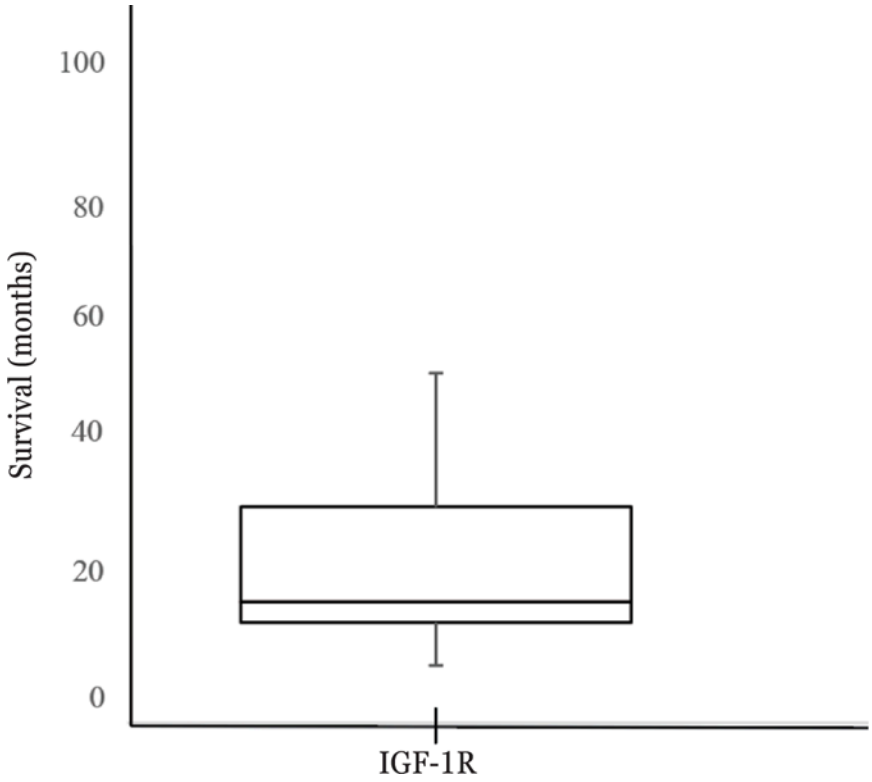

Figure 6. Survival (Months) for IGF1R in Liver Cancer.

The box-and-whisker plot is given to compare the spread of patient survival data in liver cancer. The medians are represented as the center of the plot.